A QUANTITATIVE STUDY OF THE EFFECT OF CARBON DIOXIDE UPON THE CLEAVAGE RATE OF THE ARBACIA EGG

CHARLOTTE HAYWOOD AND WALTER S. ROOT

(From the Marine Biological Laboratory, Woods Hole, Massachusetts, and the Department of Physiology, School of Medicine, University of Pennsylvania)

In two important respects carbon dioxide claims a place of peculiar interest for the physiologist. First, as a metabolic product it is present wherever there are active cells. Secondly, as many workers have observed, it has the effect of very promptly depressing certain cell activities. The exact extent of this depressant action of carbon dioxide upon the cells which produce it is an important question yet to be answered. A step in this direction, however, can be made by a determination, as quantitative as possible, of the effect of relatively low, accurately measured tensions of carbon dioxide upon some type of cell activity.

For such a study the fertilized egg of the sea urchin is especially useful, since it is less subject to wide variations than are many other cells, and since its rate of cleavage provides a convenient means of measuring any possible effects produced. Moreover, inasmuch as one of us (Root, 1930) has shown that the presence of carbon dioxide definitely limits the amount of oxygen consumed by the fertilized *Arbacia* egg, it is of interest to ascertain whether this effect of various known tensions of carbon dioxide upon cell oxidations is paralleled by its effect upon the cleavage rate.

Haywood (1927) has shown that, if carbon dioxide at a tension greater than 20 per cent of an atmosphere is added for even a few minutes to the sea water surrounding the newly fertilized eggs of *Arbacia,* cleavage suffers a distinct retardation. It might, therefore, be expected that lower tensions of the gas could also delay cleavage if administered for longer periods of time. In fact, this was earlier demonstrated by Smith and Clowes (1924) by a different method. These workers obtained the carbon dioxide from the bicarbonate of the sea water by the addition of hydrochloric acid, but with the result that the amount of bicarbonate remaining was not constant throughout this series of experiments. Since they themselves showed that the inhibitory effect of carbon dioxide is increased as the amount of bicarbonate is decreased, the results obtained by these workers are of a relatively complex nature.

In the present investigation, the newly fertilized eggs of *Arbacia* were subjected to sea water whose composition was not altered except by the addition of carbon dioxide. Moreover, we believe that our method has a further theoretical advantage because the data are based on the time required for one definite stage in the cleavage process to be reached (*i.e.*, the appearance of the first cleavage in 50 per cent of the eggs) rather than on the total number of cleavages occurring in all of the eggs in some arbitrarily selected time.

Method

Sea water containing the desired tension of carbon dioxide was prepared by equilibration with carbon dioxide-air mixtures, as described by Root (1930) elsewhere. This method consisted essentially of bubbling the gas from a mixing bottle into a tonometer containing sea water until the latter was found to be fully equilibrated with the gas, at which time duplicate gas samples were taken for subsequent analysis with a Bazett modification (1928) of the Haldane-Henderson gas analyzer. At the same time a tonometer for the control was equilibrated with room air. Our method was slightly modified from that of Root in two ways. First, both the carbon dioxide-air mixture and the room air used for equilibration were passed through a series of bottles containing sea water before entering the tonometers, to eliminate any possible change in osmotic pressure through evaporation. Secondly, the eggs were not added to the tonometers until *after* equilibration, because preliminary control experiments had indicated that the agitation of the eggs by the bubbling of the gas apparently had a retarding effect upon the rate of cleavage. Since only 0.9 cc. of egg suspension was introduced into 75–90 cc. of sea water in the tonometers which were used, it will be evident that any change in gaseous content due to the addition of the eggs was extremely small.

The lowering of the oxygen content of the carbon dioxide-air mixture caused by the addition of the carbon dioxide to room air could be entirely disregarded, for a previous study by Haywood (1927) has shown that the oxygen tension may be reduced far more than occurred in these experiments before the cleavage rate is affected by lack of oxygen.

The suspensions of eggs and sperm of *Arbacia punctulata* which were employed were freshly obtained and were freed of debris. A preliminary observation of their quality was made by fertilizing samples and noting the appearance of the fertilization membrane. The eggs were inseminated and then allowed to settle for five or six minutes. At the end of that time they were gently centrifuged by means of a

hand centrifuge and the supernatant liquid containing sperm was removed, after which the egg suspension was diluted to the desired concentration by the addition of a small amount of sea water. At exactly 10 minutes after insemination, 0.9 cc. of the resulting egg suspension was introduced into each of the two tonometers, one of which was already equilibrated at the desired temperature with a carbon dioxide-air mixture, the other, with room air. Besides the two tonometers, a glass-stoppered bottle of about 80 cc. capacity, completely filled with sea water, also received 0.9 cc. of egg suspension. This bottle was useful as a check upon the control tonometer and also allowed inspection of the eggs at times when it was not desirable to open the control tonometer.

A temperature of $20.6° \pm 0.5°$ C. was maintained by means of ice in all the experiments except those of eight hours' or more duration. In these longer experiments the variations in temperature were not more than $\pm 2.0°$ C.

After exposure to the gas of the tonometers for varying lengths of time, samples of the eggs were obtained for observation. After a preliminary precaution of cleaning the outlet tube of the tonometer with a stream of sea water, a few drops of the suspension were removed as a sample. This could be done without the admission of air by opening only the lower stop-cock while the tonometer was held in the hand for a moment, the warmth of the hand expanding the gas within the tonometer sufficiently to force out the sample. Further division of the eggs thus removed was immediately prevented by adding a weak solution of formaldehyde in sea water, which preserved them until a time when they could be accurately counted. It was, of course, necessary to take as few samples as possible in order to minimize the loss of gas from the tonometers. Four or five samples were usually sufficient to provide the requisite data, cleavage in 50 per cent of the eggs being completed in most cases before the third sample was taken. Since the samples could ordinarily be obtained within a few minutes of one another, the period during which the carbon dioxide tension was slightly lowered was a relatively short one. The maximum reduction in tension due to total sampling was never greater than 5 per cent.

The eggs were counted to determine the percentage showing the first cleavage. The counts were based upon samples of two hundred or more eggs, but occasionally it was necessary to use smaller samples. The percentages were then plotted against the time in minutes after insemination (Fig. 1). As already stated in an earlier paper (Haywood, 1927), the resulting curve theoretically represents the number of eggs which from minute to minute are undergoing the first cleavage,

and from it by interpolation may easily be determined the time required for cleavage to appear in any given percentage of eggs. The time required for the first cleavage in 50 per cent of the eggs was chosen as a convenient criterion of the rapidity of the cleavage process, and will be referred to subsequently as the *cleavage time*. A comparison of the cleavage time of the eggs of the two tonometers, therefore, gives a basis for studying quantitatively the effect of carbon dioxide upon the rate of cleavage.

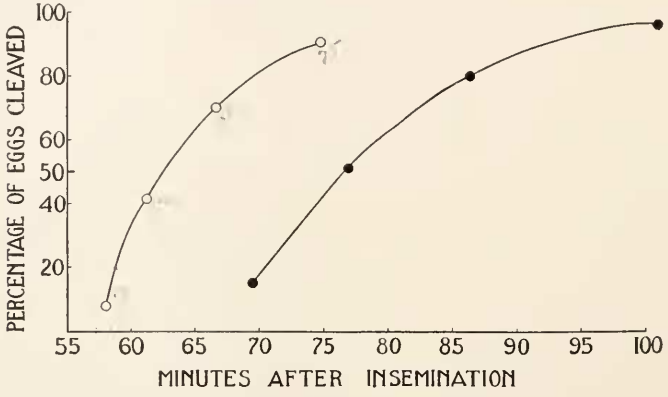

Fig. 1. The cleavage rate of *Arbacia* eggs during exposure to a carbon dioxide tension of 9.5 mm. Hg.

Abscissa = time, in minutes, after insemination.
Ordinate = percentage of eggs showing the first cleavage.
● = exposed eggs. ○ = control eggs.

Both sets of eggs were placed in the tonometers 10 minutes after insemination. Temperature = 20.6°–20.8° C.

Results

The data of a typical experiment are shown graphically in Fig. 1. From the curve at the left, the cleavage time of the control was found to be 62.5 minutes, while a similar curve, unquestionably shifted toward the right, shows the effect of 9.5 mm. Hg tension of carbon dioxide in retarding the cleavage time to 76.5 minutes. This represents a delay of 14 minutes. Since exposure to carbon dioxide was not begun until 10 minutes after insemination, it must be realized that in every case the time of exposure was actually 10 minutes less than the time of cleavage. Thus, in the experiment cited, the 76.5 minutes measured as the cleavage time really represent but 66.5 minutes of exposure, and hence the value of 14 minutes given as the delay for the carbon dioxide tension employed may differ slightly from that which would

have been obtained had the exposure been started immediately after fertilization. At the higher tensions of carbon dioxide, the curves obtained were somewhat flatter, and the number of eggs ultimately cleaving was often not as great. Abnormalities of cleavage were also to be found at some of the tensions employed. In such cases, the abnormally cleaved eggs were counted separately from the normal ones, but the total number of cleavages, abnormal as well as normal, was used in determining the cleavage time.

By means of sets of cleavage curves, obtained as were those in Fig. 1, the retardation of cleavage was determined for twenty-five different tensions of carbon dioxide, covering a range of from 1.8 mm. Hg to 44.0 mm. Hg. Some variation is naturally to be expected, especially since the range of carbon dioxide tensions employed was a relatively narrow one. Nevertheless, the general trend of the data is wholly obvious, as may be seen by observing Fig. 2. The pH values were taken from the data of Henderson and Cohn (1916). Tensions of as low as 4 mm. were able to retard cleavage 9 minutes, while one of 15 mm., which corresponds to about 2 per cent carbon dioxide, delayed it 16 minutes or more. With higher tensions of carbon dioxide, up to about 35 or 40 mm., the retardation was correspondingly greater. At tensions of 35 or 40 mm. and above, small increments of the gas were found to inhibit cleavage to a degree which was proportionally much greater than at lower tensions, and the curve, therefore, ascends steeply from this point on.

Whatever may be the importance of this critical tension of carbon dioxide, the effectiveness of very small amounts of the gas in retarding cleavage seems of real significance. Since tensions of the gas corresponding to 1 or 2 per cent—values which may conceivably approach carbon dioxide concentrations under conditions of crowding—are capable of causing an easily observed delay, it is apparent that the cells are highly sensitive to this gas.

The effects of carbon dioxide upon cell division appear quantitatively quite similar to those upon cell oxidations. This may readily be seen by comparing our curve in Fig. 2 with that obtained by one of us (Root, 1930) when the oxygen consumption of the eggs is plotted against the carbon dioxide tensions of the medium. It should be noted that both curves bend sharply at relatively the same carbon dioxide tension,—namely, at 35–40 mm. Hg. Apparently carbon dioxide acts upon some system within the cell which results in a depressed rate of oxygen consumption and an increased cleavage time.

As was mentioned earlier, when eggs were found to have divided abnormally in solutions containing carbon dioxide, the total number

of cleavages, abnormal as well as normal, was used in plotting the cleavage curves. Therefore a different set of symbols was used in Fig. 2 to designate cleavage times in eggs where abnormalities resulted from exposure to the carbon dioxide. An examination of the relative number of abnormal and normal cleavages occurring at twelve different carbon dioxide tensions between 25.6 and 49.4 mm. Hg inclusive, showed the average ratio of abnormal to normal cleavages to be 2.5. The ratios ranged from 1 to 5.5 and tended to be somewhat higher for the higher carbon dioxide tensions.

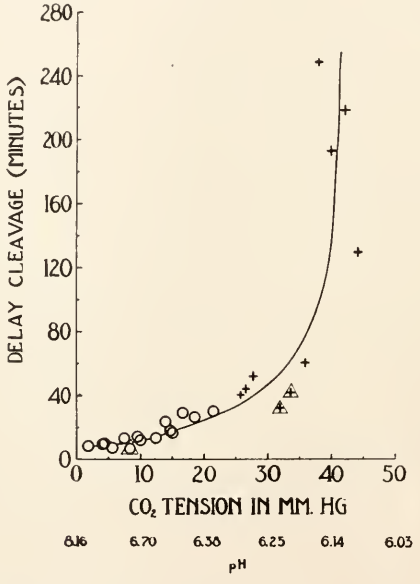

Fig. 2. The retardation of the first cleavage of *Arbacia* eggs during exposure to various carbon dioxide tensions.
Abscissa = carbon dioxide tension in mm. Hg; pH is also given.
Ordinate = minutes of retardation of the first cleavage.
○ = cleavages normal in at least 95 per cent of the cleaved eggs.
+ = abnormal cleavages included.
△ = values approximate only, since these were obtained by extrapolation.
Temperature = 20.6° ± 0.5° C.

After determining the extent to which cleavage was retarded by relatively low tensions of carbon dioxide, we next undertook to ascertain the tension of this gas which would be required to suppress cleavage completely. In these experiments, the eggs were exposed to carbon dioxide for eight hours or more, at the end of which time samples were taken and the percentage of cleaved eggs noted. Two controls were always carefully made in these experiments: First, a count was

made of eggs which had been fixed in formaldehyde soon after fertilization, in order to make sure that no cleaved eggs were accidentally present; secondly, a count was made of eggs which had been kept for at least an hour in ordinary sea water, in order to determine whether or not the eggs used were fully capable of undergoing division. If the exposed eggs showed no cleavage in eight hours, the suppression of cleavage was regarded as complete. Such completely suppressed eggs were found to show no abnormalities in appearance, but retained completely the appearance of newly fertilized eggs. The results obtained are shown in Fig. 3, where the percentage of eggs cleaved is plotted against the tension of carbon dioxide used. Although variations in the results obtained with different groups of eggs make it impossible to designate any one tension of carbon dioxide as the threshold for the complete suppression of cleavage, the data are sufficiently consistent to allow a tentative estimate of 120 to 130 mm. Hg carbon dioxide for this value. This would correspond to 16 to 17 per cent carbon dioxide at 760 mm. Hg and 20.6° C., or a solution one-sixth saturated with carbon dioxide.

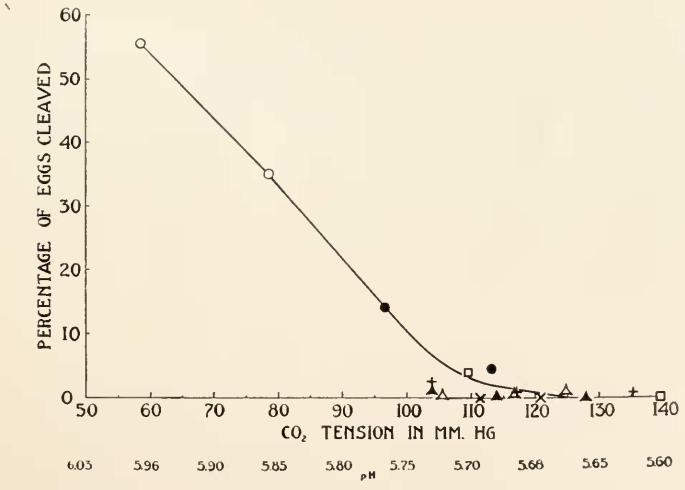

FIG. 3. The point of complete suppression of *Arbacia* eggs with carbon dioxide.

Abscissa = carbon dioxide tension in mm. Hg; pH is also given.

Ordinate = percentage of eggs showing the first cleavage after exposure of eight hours or more to carbon dioxide. Abnormals as well as normals are included.

The various symbols represent the different egg suspensions used.

Temperature = 20.6° ± 2.0° C.

This value of 16 to 17 per cent carbon dioxide, which is required for total inhibition of cleavage, is in wide disagreement with the 3.8 per cent obtained by Smith and Clowes (1924) at 20.0° C. It is important to realize, however, that the method of these investigators of liberating carbon dioxide from the bicarbonate of sea water by adding strong acid introduces a second variable—a varying bicarbonate content. These authors point out that, with a diminished bicarbonate content, the effect of carbon dioxide is more pronounced. The experimental conditions of Smith and Clowes' work differ sufficiently from those of ours to make difficult an agreement between the two sets of values.

Summary

1. Sea water equilibrated with analyzed carbon dioxide-air mixtures was found to retard the first cleavage of *Arbacia* eggs which were introduced into the mixture ten minutes after insemination.

2. A measurable delay of cleavage occurred at tensions of carbon dioxide of as low as 4 mm. Hg. Retardation was progressively greater up to 120–130 mm. Hg, at which point complete suppression occurred.

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